



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07J 71/00, A61K 31/56 // C07J 3/00, 5/00	A1	(11) International Publication Number: WO 94/13690 (43) International Publication Date: 23 June 1994 (23.06.94)
(21) International Application Number: PCT/GB93/02537 (22) International Filing Date: 13 December 1993 (13.12.93) (30) Priority Data: 9225923.3 11 December 1992 (11.12.92) GB (71) Applicant (for all designated States except US): RHONE-POULENC RORER LIMITED [GB/GB]; RPR House, St Leonards Road, Eastbourne, East Sussex BN21 3YG (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): ASHTON, Michael, John [GB/GB]; Rhone-Poulenc Rorer Limited, Dagenham, Essex RM10 7XS (GB). WITHNALL, Michael, Thomas [GB/GB]; Rhone-Poulenc Rorer Limited, Dagenham, Essex RM10 7XS (GB). KARLSSON, Sven, Jan-Anders [SE/GB]; Rhone-Poulenc Rorer Limited, Dagenham, Essex RM10 7XS (GB). VACHER, Bernard, Yvon, Jack [FR/GB]; Rhone-Poulenc Rorer Limited, Dagenham, Essex RM10 7XS (GB). (74) Agents: BENTHAM, Stephen et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: NEW STEROIDS (57) Abstract <p>Steroids of formula (I) where \equiv is a single or double bond; R¹ is hydrogen or fluorine; and R² is propyl or trans-prop-1-enyl, and racemic mixtures and diastereoisomers thereof, processes for their preparation, pharmaceutical compositions containing them, and methods for their use, especially as anti-inflammatories.</p> <div style="text-align: center;"> <p style="text-align: right;">(I)</p> </div>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

"NEW STEROIDS"FIELD OF THE INVENTION

The present invention relates to novel antiinflammatory, immunosuppressive, and antiallergic compounds and to processes
5 for their preparation. The invention also relates to pharmaceutical compositions containing the compounds. The invention also relates to the pharmacological uses of the compounds.

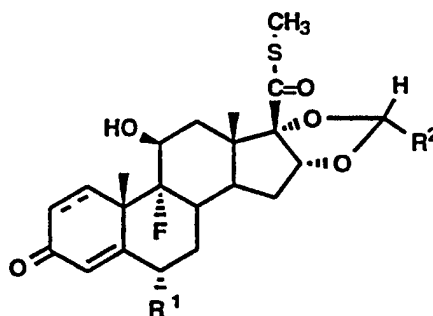
More particularly, this invention relates to new
10 therapeutically useful steroids, processes for their preparation, pharmaceutical compositions containing them, and methods for their use, especially as antiinflammatories.

The object of the invention is to provide a steroid which possesses high antiinflammatory, immunosuppressive and
15 antiallergic activity, or a pharmaceutical composition thereof, with high activity at the site of application, e.g. in the respiratory tract, on the skin, in the joints, in the intestinal tract, or in the eye, coupled with low glucocorticoid systemic potency.

20 A large number of natural and synthetic steroids are known, and many of them are useful in the treatment of human and animal subjects. Steroids which have antiinflammatory properties are known, but they suffer from the disadvantage that, after administration, they cause unwanted side-effects
25 outside the organ or tissue which is desired to be treated. It is well known that, in the pharmaceutical field and, in particular, in the field of steroids, small differences in chemical structure can produce compounds with completely

different pharmacological activities. The present invention provides compounds which have never been described hitherto and which possess a remarkable combination of very useful antiinflammatory activity with a very low ability to produce
 5 undesired side-effects, to an extent which has never before been found in steroid compounds.

The compounds of this invention may be described by general formula I



I

where:

--- is a single or double bond;

15 R¹ is hydrogen or fluorine; and

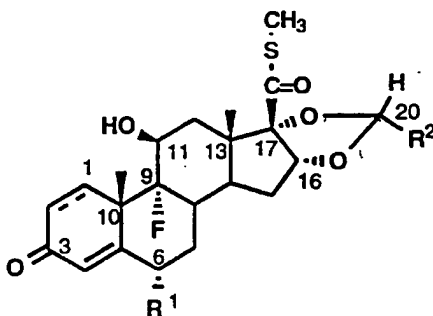
R² is propyl or trans-prop-1-enyl and
 racemic mixtures and diastereoisomers thereof.

DETAILED DESCRIPTION AND PREFERRED EMBODIMENTS

The nomenclature used in this application is as follows:

20

3



The 1,2-position is saturated or is a double bond

The more preferred compounds are those where --- is a

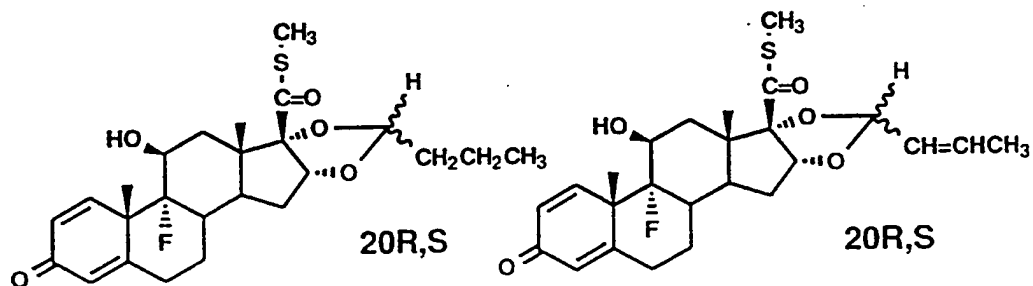
5 double bond.

Each of the compounds of formula I can exist in two diastereoisomeric forms because two different configurations are possible at the carbon atom in the 20 position.

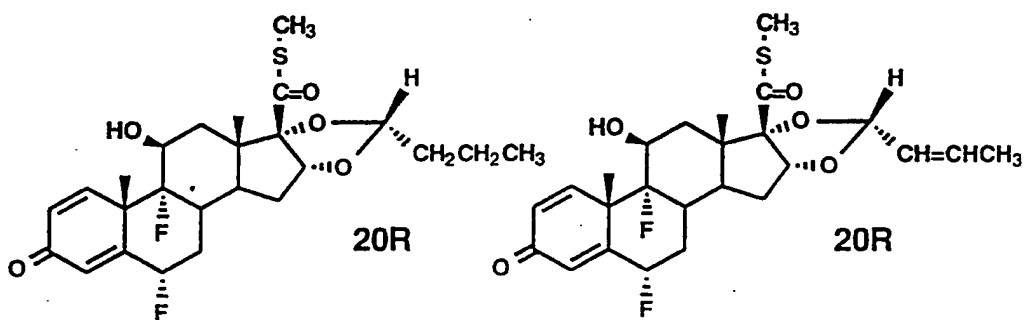
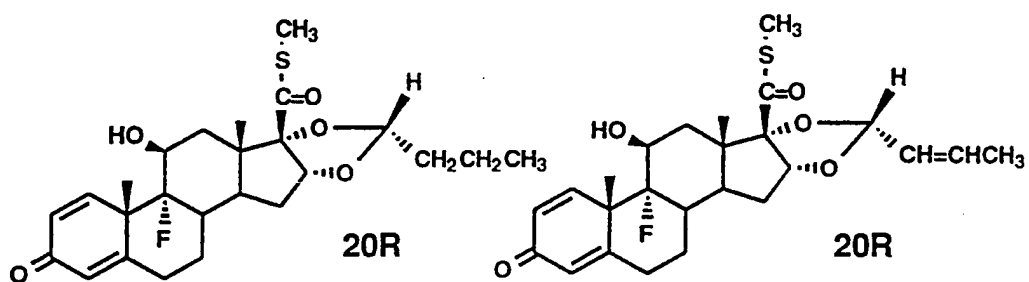
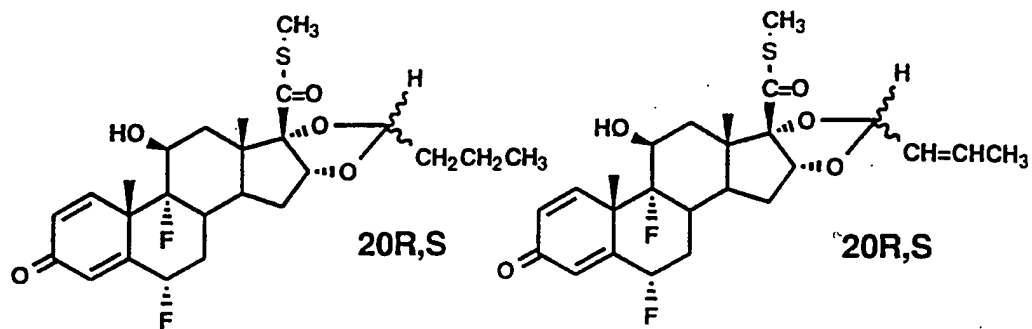
As a result, the invention includes the (20R)- and
 10 (20S)-diastereoisomers of the compounds of formula I, and mixtures thereof. The preferred diastereoisomeric components are in the (20R)-configuration.

More specifically, the following compounds are within the scope of this invention.

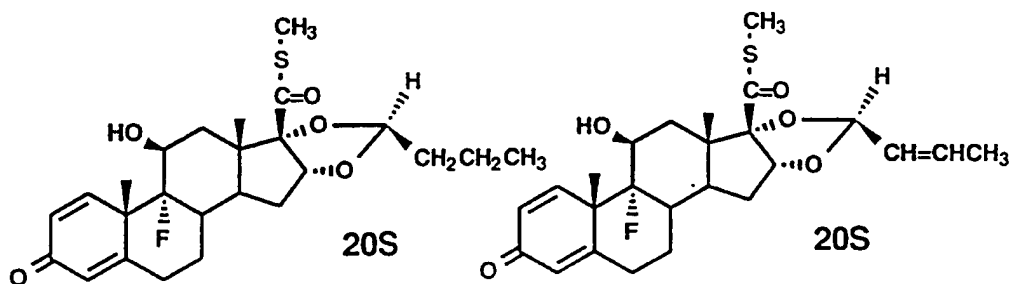
15

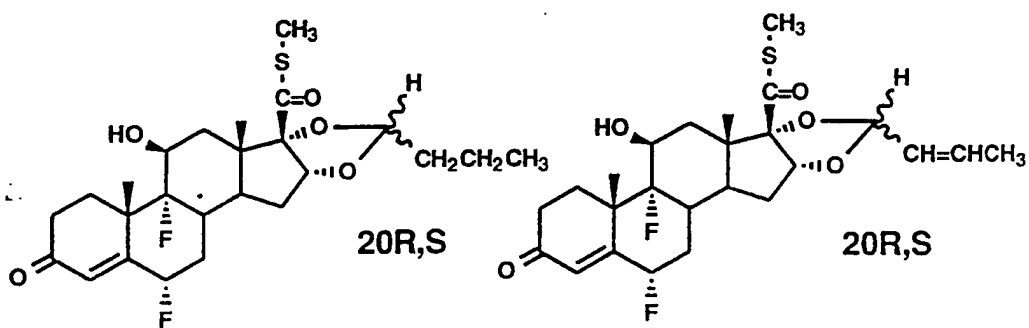
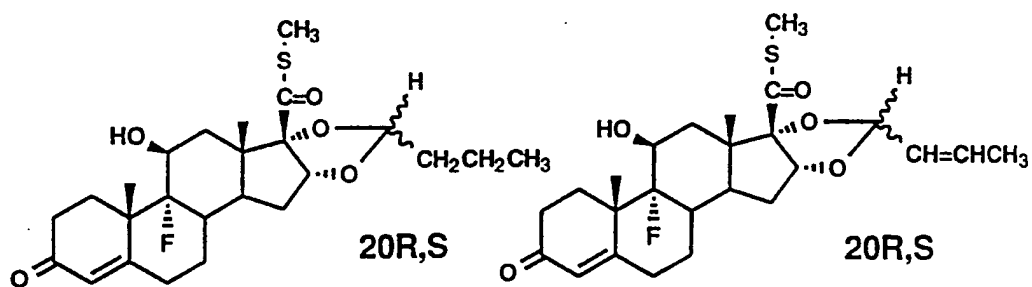
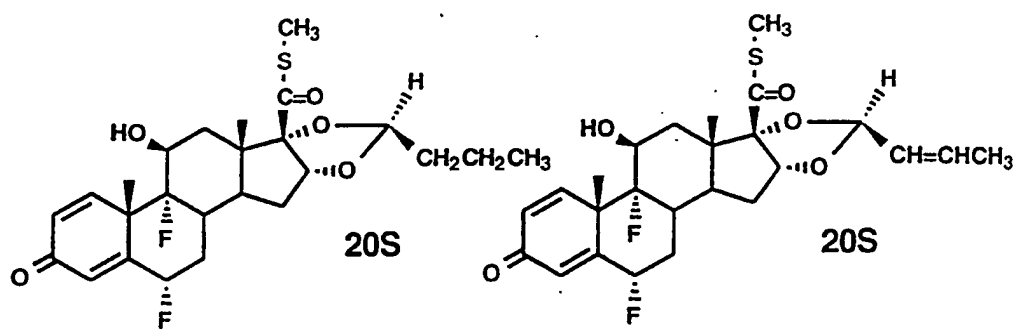


4

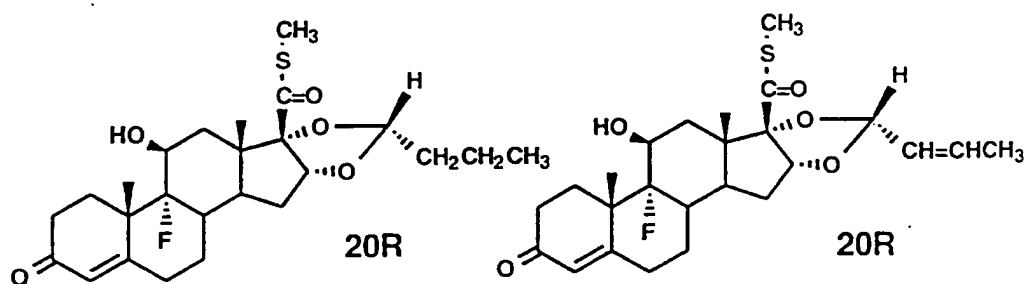


5



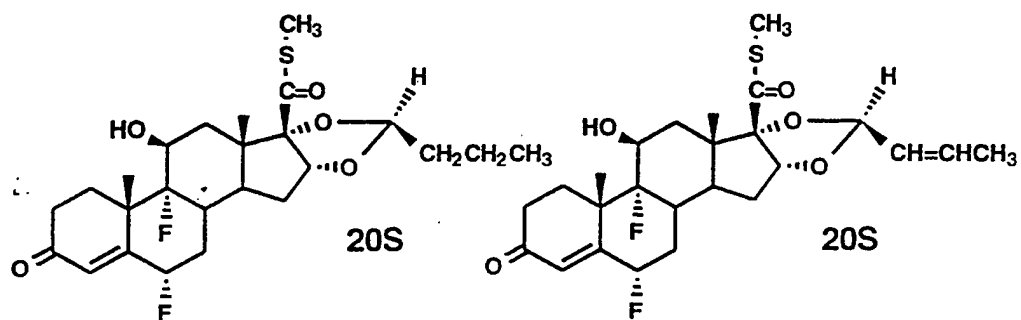
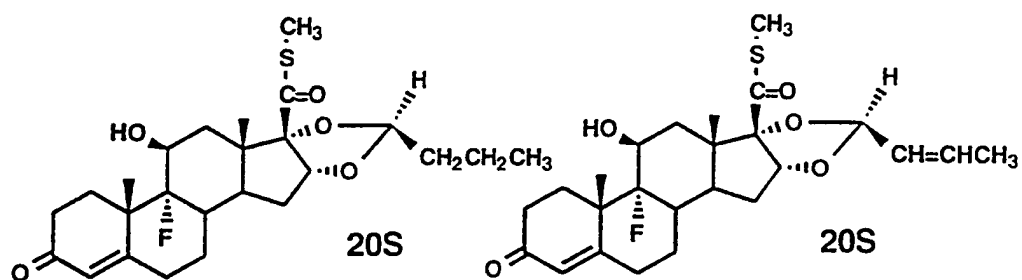
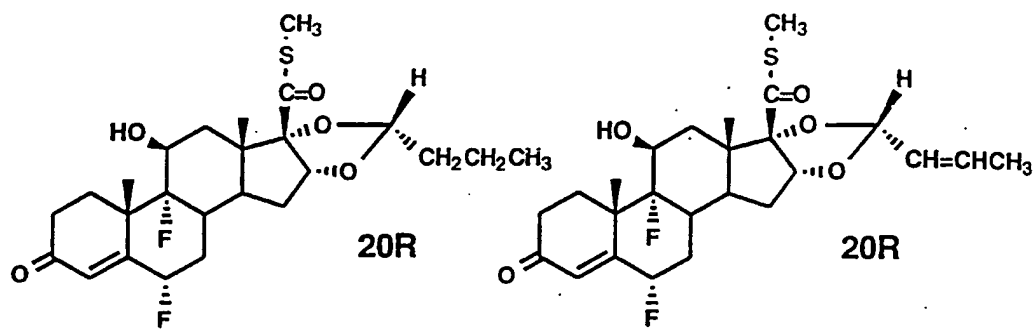


5



SUBSTITUTE SHEET

6



5

SUBSTITUTE SHEET

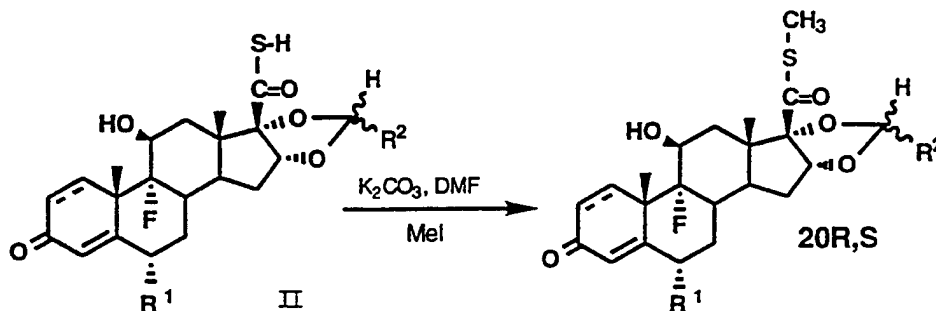
The compounds are extremely valuable in the local treatment of inflammatory, allergic and immunological diseases. These include those currently treated by known steroids, such as diseases of the respiratory system, e.g. asthma and rhinitis, diseases of the skin, e.g. eczema, and diseases of the gastrointestinal tract, e.g. inflammatory bowel disease. However, because of the advantage of having little or no side effects, the compounds of the invention are much more desirable than any previously known compounds.

10 The use of the compounds of formula I, and of pharmaceutical formulations containing them in the treatment of such diseases, form features of the present invention.

These utilities have been demonstrated in pharmacological tests which are believed to correlate well to activity in humans and other mammals.

The compounds of formula I can be prepared by the application or adaptation of known methods, by which is meant methods used hitherto or described in the literature.

The compounds of this invention may be prepared, for example, by the following reactions.

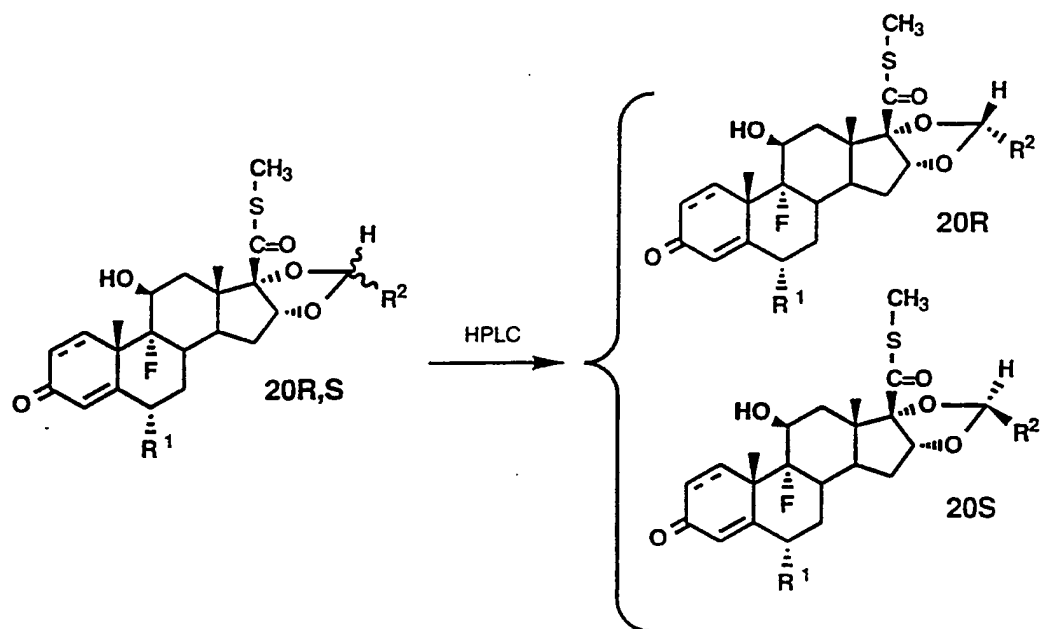


Thus, according to a feature of the present invention, compounds of formula I are prepared by the methylation of compounds of the general formula II, hereinbefore depicted, wherein --- , R^1 and R^2 are as hereinbefore defined, by known methods, for example by reaction with a base followed by reaction with methyl iodide.

According to a further feature of the present invention, compounds of formula I are prepared by the reaction of compounds of the general formula III, hereinafter depicted, wherein --- , R^1 and R^2 are as hereinbefore defined, and -COX represents a group of formula IV hereinafter depicted, wherein R^3 is as hereinafter defined, with methanethiol or an alkali metal salt thereof, e.g. sodium methanethiolate.

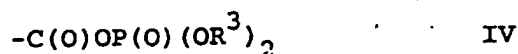
The diastereoisomers of general formula I can be separated from their mixtures, by the application or

adaptation of known methods, for example chromatographic and recrystallisation techniques, or they may be separately prepared from the appropriate isomers of their intermediates, for example by the application or adaptation of methods
 5 described herein.



The starting materials and intermediates can be prepared
 10 by the application or adaptation of known methods, for example methods described in the Examples or their obvious chemical equivalents. For example, compounds of formula II can be prepared by the reaction of an alkali metal hydrosulphide, e.g. sodium hydrosulphide, with a compound of the general
 15 formula III, hereinafter depicted, wherein --- , R^1 and R^2 are as hereinbefore defined and -COX is an active derivative

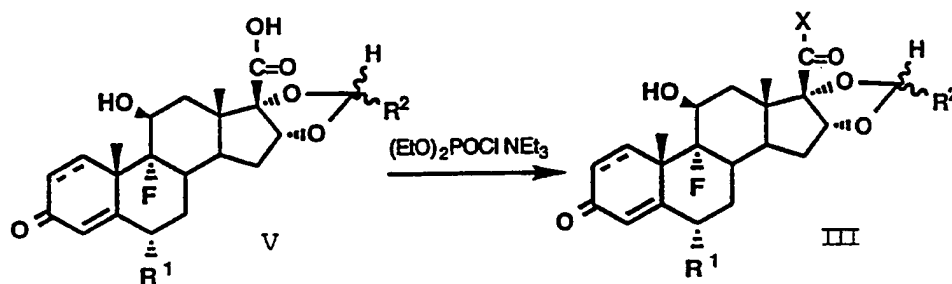
of a carboxy group, for example a group of the general formula:-



wherein R^3 represents an alkyl, preferably ethyl, group.

- 5 Compounds of formula III can be prepared from the corresponding carboxylic acids of the general formula V, hereinafter depicted, wherein --- , R^1 and R^2 are as hereinbefore defined, by the application or adaptation of known methods.

10



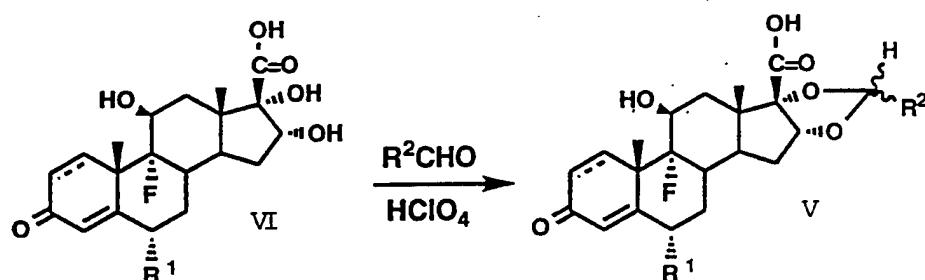
- Compounds of formula V can be prepared by the application or adaptation of known methods, for example from compounds of the general formula VI, hereinafter depicted, wherein --- and R^1 are as hereinbefore defined, by reaction with compounds of the general formula:-



- wherein R^2 is as hereinbefore defined, in the presence of
20 perchloric acid.

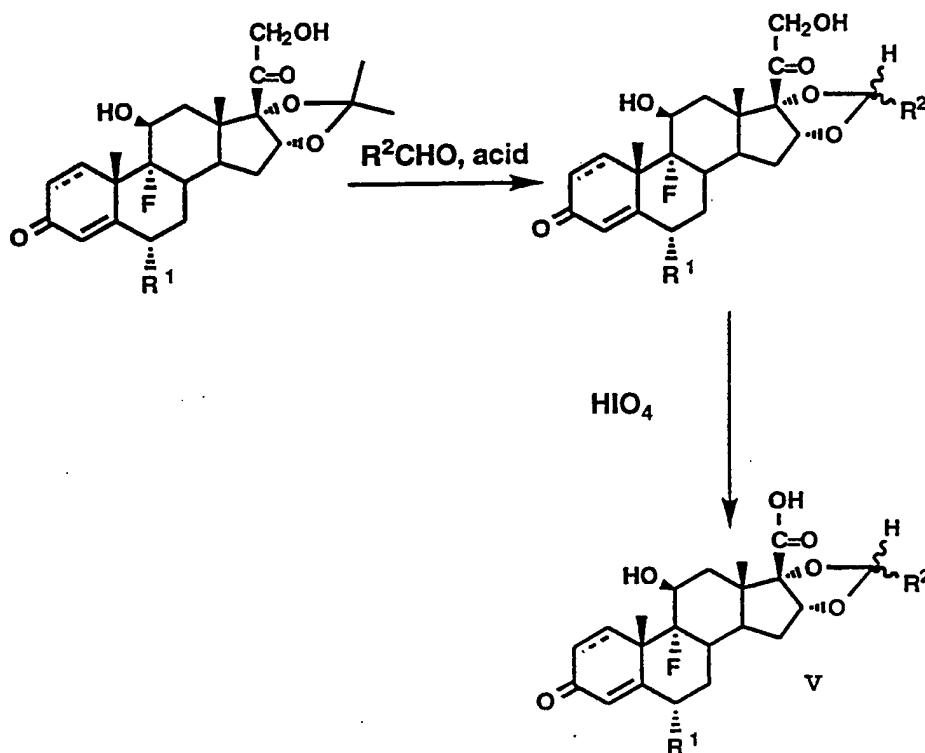
SUBSTITUTE SHEET

11



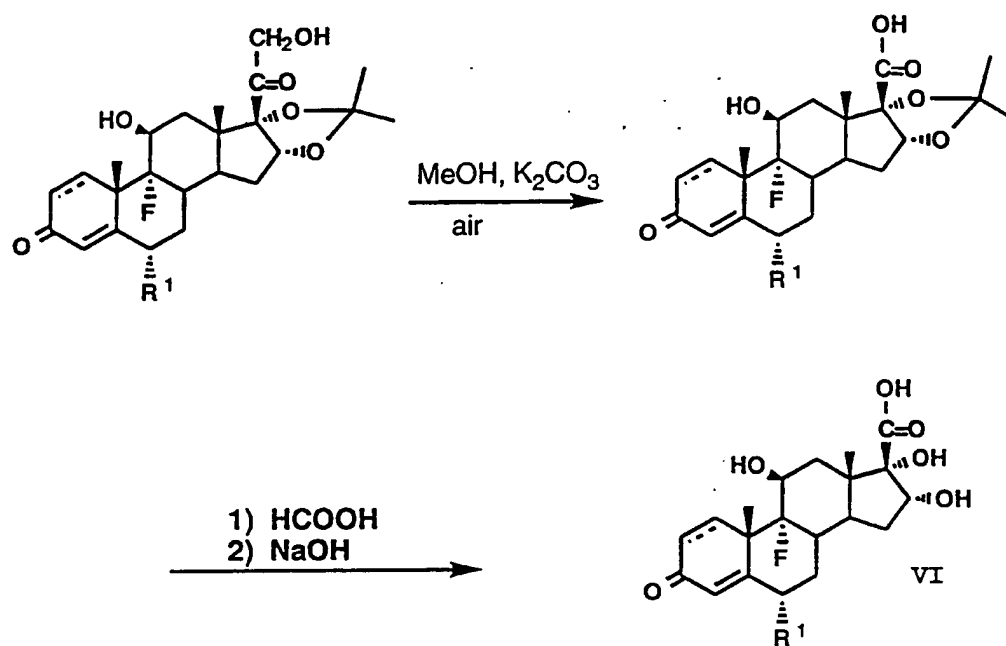
Alternatively, compounds of formula V can be prepared by the following reaction sequence.

5



Compounds of formula VI can be prepared by known methods such as the following reactions.

10



SUBSTITUTE SHEET

Methods of preparation

The following Examples illustrate the preparation of compounds according to the present invention. All $^1\text{H-NMR}$ spectra are recorded at 400MHz. The chemical shifts are expressed in ppm relative to tetramethylsilane. Abbreviation in the text have the following significances: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, c = unresolved complex peak, b = broad signal. Optical rotations are measured using a polarimeter model AA-10.

EXAMPLE 1

S-Methyl (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate

A (20R,S)-16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid

To a stirred suspension of 6 α ,9 α -difluoro-11 β ,16 α ,17 α -trihydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid (50.0g, 126mmol) in tetrahydrofuran (2l) at 25°C under a nitrogen atmosphere is added butyraldehyde (59.2g, 820mmol) and perchloric acid (1.2g, 11.8mmol). The reaction mixture is stirred for 16 hours and then treated dropwise with triethylamine (1.2g, 11.8mmol). Evaporation of the solvent in vacuo gives a yellow oil which is partitioned between ethyl acetate (1 litre) and sodium carbonate (2N). The aqueous phase is decanted, washed with more ethyl acetate (400ml), acidified to pH 2 with hydrochloric acid (10N) before being extracted with diethyl ether (1 litre). The combined diethyl ether extracts are washed with water and then with brine, and

SUBSTITUTE SHEET

then dried over magnesium sulfate. Filtration of the desiccant and evaporation of the solvent in vacuo gives a white solid which is triturated with cyclohexane then filtered off and dried in vacuum at 80°C overnight to obtain

5 (20R,S)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid (54.8g, 121mmol);

[N.M.R. (DMSO, d₆): 0.85 (m, 3H), 0.96 (s, 3H), 1.22-1.46 (c, 4H), 1.50 (s, 3H), 1.49-1.62 (m, 3H), 1.79 (c, 1H), 1.90-2.06 (m, 3H), 2.26 (c, 1H), 2.45-2.67 (m, 1H), 4.17 (c, 1H), 4.68

10 (t, 0.8H), 4.88 (d, 0.8H), 5.10 (d, 0.2H), 5.19 (t, 0.2H), 5.45 (d, 1H), 5.63 (c, 1H), 6.10 (s, 1H), 6.29 (dd, 1H), 7.35 (d, 1H)]. This compound is used in the next step without further purification.

B (20R,S)-16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β -

15 hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic

diethyl phosphoric anhydride

A stirred suspension of (20R,S)-16 α ,17 α -butylidene-dioxy-6 α ,9 α - difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid (14.5g, 32mmol) in tetrahydrofuran (400ml)

20 containing activated molecular sieve (type 4A, 4g) and maintained under a nitrogen atmosphere is treated dropwise with triethylamine (6.5g, 64mmol). After stirring for 20 minutes, diethyl chlorophosphate (10.6g, 61.6mmol) is added during 10 minutes to the reaction mixture and stirring is

25 continued for a further 2 hours. The resulting mixture is filtered through a pad of diatomaceous earth to remove the precipitate of triethylammonium chloride, and then the solvent is evaporated off in vacuo. The crude yellow oil obtained is

dissolved in ethyl acetate (500ml), washed with hydrochloric acid (1N, 250ml) then with water (two times 250ml) and finally with brine (two times 200ml). The ethyl acetate phase is dried over magnesium sulfate, the desiccant is then filtered off and evaporation of the solvent in vacuo gives
5 (20R,S)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic diethyl phosphoric anhydride, in the form of a pale yellow gum (19.5g crude) which is used as such in the next step.

10 C (20R,S)-16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioic acid

A stirred solution of sodium hydrosulfide (11.3g) in ethanol (215ml) and water (16ml) at 25°C under a nitrogen atmosphere is treated with a solution of the crude
15 (20R,S)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic diethyl phosphoric anhydride (19.0g) in tetrahydrofuran (250ml). The reaction mixture is stirred for 1 hour then diluted with water (500ml) and concentrated in vacuo to remove most of the organic
20 solvents. The remaining aqueous phase is treated with water until its volume is 500ml and then it is washed with diethyl ether (500ml). The ethereal washings are discarded. The aqueous phase is acidified to pH 2 by treatment with hydrochloric acid (10N) and then it is extracted thoroughly
25 with diethyl ether. The combined diethyl ether extracts are washed with water (200ml) and with brine (two times 200ml) and then dried over magnesium sulfate. Filtration of the desiccant and evaporation of the solvent in vacuo gives

(20R,S)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioic acid (14.7g), in the form of an off-white solid, which is used as such in the next step.

5 D S-Methyl (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate

A stirred solution of the crude (20R,S)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioic acid (14.7g, 31.3mmol) in
10 dimethylformamide (240ml) under a nitrogen atmosphere is treated with anhydrous potassium carbonate (5.6g, 40mmol) at 25°C. After 30 minutes stirring, the dark red solution obtained is treated with methyl iodide (6.9g, 48.5mmol) and the resulting bright yellow solution stirred for a further 1
15 hour. The reaction mixture is added to an ice cold hydrochloric acid solution (0.1N, 1.2l). The resulting white precipitate is collected by filtration, washed with cold water (100ml) then dissolved in ethyl acetate (400ml). The ethyl acetate phase is washed with water (two times 200ml), then
20 with brine (two times 200ml) and dried over magnesium sulfate. After the desiccant is filtered off, the solvent is removed under vacuo to give a white solid (14.2g) from which the mixture of epimers (20R,S) in proportion of 80% to 20% is resolved by preparative HPLC using a Dynamax RP-18 column and
25 acetonitrile/water as the mobile phase.

The S-methyl (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate is obtained in a purity greater than 98%, m.p. 245-6°C;

$[\alpha]^{26} = +160^\circ$, $c = 0.065$ (CH_3CN); [N.M.R. (DMSO, d_6): 0.88 (t, 6H), 1.36 (m, 2H), 1.43 (dd, 1H), 1.49 (s, 3H), 1.50-1.67 (m, 4H), 1.70 (d, 1H), 1.95-2.08 (m, 2H), 2.23-2.30 (c, 1H), 2.25 (s, 3H), 2.50-2.69 (m, 1H), 4.18 (c, 1H), 4.65 (t, 1H), 4.75 (d, 1H), 5.49 (c, 1H), 5.64 (c, 1H), 6.10 (s, 1H), 6.30 (dd, 1H), 7.25 (dd, 1H);

Found: C, 62.4; H, 6.66%

Calculated for $\text{C}_{25}\text{H}_{32}\text{F}_2\text{O}_5\text{S}$: C, 62.2; H, 6.68%].

EXAMPLE 2

10 S-Methyl (20R)-16 α ,17 α -[(E)-2-butenylidenedioxy]-9 α -
fluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate
 A (20R,S)-16 α ,17 α -[(E)-2-Butenylidenedioxy]-9 α -fluoro-
11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid

In an analogous manner following the procedure of Example
 15 1, Step A but using 9 α -fluoro-11 β ,16 α ,17 α -trihydroxy-3-
 oxoandrosta-1,4-diene-17 β -carboxylic acid (8.9g, 23.4mmol) and
 crotonaldehyde (7.4g, 105.3mmol) as starting materials gives
 after work up (20R,S)-16 α ,17 α -[(E)-2-butenylidenedioxy]-9 α -
 fluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid
 20 (9.1g, 21mmol); [N.M.R. (DMSO, d_6): 0.96 (s, 3H), 1.30-1.43
 (m, 1H), 1.50 (s, 3H), 1.47-1.60 (m, 2H), 1.64 (dd, 0.6H),
 1.68 (dd, 2.4H), 1.68-2.0 (m, 5H), 2.34 (dd, 1H), 2.35-2.52
 (m, 1H), 2.64 (dt, 1H), 4.15 (c, 1H), 4.85 (d, 0.8H), 4.97 (d,
 0.8H), 5.07 (d, 0.2H), 5.28 (ddd, 0.2H), 5.33-5.40 (m, 1.8H),
 25 5.46 (d, 0.2H), 5.85 (m, 0.2H), 5.92 (m, 0.8H), 6.03 (s, 1H),
 6.23 (dd, 1H), 7.29 (d, 1H)]. This compound is used as such
 in the next step.

B (20R,S)-16 α ,17 α -[(E)-2-Butenylidenedioxy]-9 α -fluoro-
11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic
diethyl phosphoric anhydride

In an analogous manner following the procedure of Example
5 1, Step B but using (20R,S)-16 α ,17 α -[(E)-2-butenylidene-
dioxyl]-9 α -fluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -
carboxylic acid (10.0g, 23.1mmol) and diethyl chlorophosphate
(6.0g, 34.7mmol) as starting materials gives after work up a
yellow oil of (20R,S)-16 α ,17 α -[(E)-2-butenylidenedioxy]-9 α -
10 fluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic
diethyl phosphoric anhydride (13g crude) which is used without
further purification in the next step.

C (20R,S)-16 α ,17 α -[(E)-2-Butenylidenedioxy]-9 α -fluoro-
11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioic
15 acid

In an analogous manner following the procedure of Example
1, Step C but using (20R,S)-16 α ,17 α -[(E)-2-butenylidene-
dioxyl]-9 α -fluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -
carboxylic diethyl phosphoric anhydride (12.5g) gives after
20 work up an off-white solid of (20R,S)-16 α ,17 α -[(E)-2-
butenylidenedioxy]-9 α -fluoro-11 β -hydroxy-3-oxoandrosta-1,4-
diene-17 β -carbothioic acid (6.6g), which is used without
further purification in the next step.

D S-Methyl (20R)-16 α ,17 α -[(E)-2-butenylidenedioxy]-9 α -
25 fluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -
carbothioate

In an analogous manner following the procedure of Example
1, Step D but using (20R,S)-16 α ,17 α -[(E)-2-butenylidenedioxy]-

9 α -fluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioic acid (15.7g, 34.9mmol) as starting material gives 19.8g of crude product which is recrystallised in ethyl acetate to give 11.0g of S-methyl (20R,S)-16 α ,17 α -[(E)-2-butenylidenedioxy]-

5 9 α -fluoro-11 β - hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate in a purity of 95-97% and with an epimer proportion of 75/25% to 80/20%. The mixture of epimers is resolved by HPLC using a porous graphitic carbon column and acetonitrile as the mobile phase, to obtain S-methyl

10 (20R)-16 α ,17 α -[(E)-2-butenylidenedioxy]-9 α -fluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate, m.p. 287-9°C; $[\alpha]^{26} = +112^\circ$, $c = 0.067$ (CH₃CN); [N.M.R. (DMSO, d₆): 0.90 (s, 3H), 1.32-1.43 (m, 1H), 1.49 (s, 3H), 1.52-1.65 (m, 2H), 1.66-1.72 (c, 1H), 1.69 (dd, 3H), 1.82 (m, 1H), 1.97 (m,

15 2H), 2.26 (s, 3H), 2.45 (dd, 1H), 2.40-2.55 (m, 1H), 2.63 (dt, 1H), 4.18 (c, 1H), 4.27 (d, 1H), 4.95 (d, 1H), 5.39 (m, 2H), 5.95 (m, 1H), 6.03 (s, 1H), 6.22 (dd, 1H), 7.28 (d, 1H);

Found: C, 64.7; H, 6.74%

Calculated for C₂₅H₃₁FO₅S: C, 64.9; H, 6.75%].

20

EXAMPLE 3

(20R)-16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid

A 16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione

25

Aqueous perchloric acid solution (75ml;70%w/v) is added rapidly to a vigorously stirred suspension of fluocinolone acetonide (100g) in heptane (2.25l) containing butanal (30ml) under an atmosphere of nitrogen. The suspension is stirred

SUBSTITUTE SHEET

at ambient temperature for 5.5 hours. The solvent is decanted and the residue is washed with fresh heptane (250ml) and decanted. The residue is then partitioned between water (1.2l) and dichloromethane (2.5l). The organic solution is removed and washed sequentially with aqueous potassium carbonate solution (500ml; 10%w/v) and water (2 x 500ml), then dried over magnesium sulphate, filtered and concentrated under reduced pressure to give 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione (103.6g; about 10:1R:S at C₂₂) in the form of a solid.

B 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid

A mixture of 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione (103.2g) and methanol is stirred under nitrogen at ambient temperature for 40 minutes to give a cloudy solution. A solution of periodic acid dihydrate (100g) in water (125ml) is added during 30 minutes, keeping the temperature below 25°C. The solution is stirred for a further 1.5 hours and is then diluted with water (2 litres). The precipitate is collected, washed with water (2 x 600ml), then suspended in water (1 litre) and 2N sodium hydroxide solution is added to pH 10 (110ml). The aqueous solution is washed with ethyl acetate (400ml, 300ml) then stirred and 2N sulphuric acid is slowly added to pH 2. The precipitate is collected, washed with water and dried to give 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid (66.7g; about 20:1 R:S at C₂₂).

SUBSTITUTE SHEET

EXAMPLE 4

16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid

A 16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione

5 Aqueous perchloric acid solution (75ml;70%w/v) is added rapidly to a vigorously stirred suspension of fluocinolone acetonide (100g) in dichloromethane (1.5l) under an atmosphere of nitrogen, then a solution of butanal (22ml) in
10 dichloromethane (500ml) is added during 8 hours. The reaction mixture is stirred at ambient temperature for 30 minutes, then washed with water (2 x 200ml) and with aqueous potassium carbonate (250ml; 10% w/v). The organic solution is dried over magnesium sulphate, filtered and concentrated
15 under reduced pressure to give 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione (101.6g; about 8.8:1 R:S at C₂₂) in the form of a solid.

B 16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid

20 A mixture of 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione (58.0g; about 8.8:1 R:S at C₂₂) and dichloromethane (580ml) is stirred under nitrogen at ambient temperature for 15 minutes to give a solution. A solution of periodic acid dihydrate (77.33g) in
25 water (155ml) is added during 5 minutes, keeping the temperature below 25°C, then triethylamine (25.25g) is added during 5 minutes. The mixture is warmed and stirred at reflux for 6 hours, then cooled and stirred at ambient

temperature overnight. tert-Butyl methyl ether (580ml) is added, followed by 2M sulphuric acid (120ml), and the mixture is stirred for 15 minutes. The organic solution is separated and 1M aqueous sodium hydroxide solution (232ml) is slowly added with stirring to pH 12. Water (250ml) is added, the mixture is stirred for 15 minutes and then the organic layer is removed. The aqueous fraction is washed with dichloromethane (250ml) and separated, and then 2M sulphuric acid (100ml) is added to pH 2. The precipitate is collected, washed with water (2 x 100ml) and dried, to give 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid (42.8g; about 89:11 R:S at C₂₂).

The dichloromethane solution of 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione obtained in Example 4A above can be used directly in the oxidation step in Example 4B without prior drying or isolation of solid material.

20

EXAMPLE 5

(20R)-16 α ,17 α -Butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid

A suspension of 16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid (47.5g; about 89:11 R:S at C₂₂) in water (950ml) is heated with stirring to 65°C. Acetone (570ml) is added slowly

maintaining the temperature between 65 and 70°C. The solution is left to cool to ambient temperature then the solid product is collected, washed with water (2 x 100ml) and dried, to give (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid (33.68g; about 98:2 R:S at C₂₂).

EXAMPLE 6

S-Methyl (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate

10 Triethylamine (15.5ml) is added to a stirred solution of (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carboxylic acid (50.0g) in dry tetrahydrofuran (1 litre) under nitrogen. After stirring for 20 minutes, diethyl chlorophosphate (25.0g) is added.

15 The mixture is stirred for 2 hours, then aqueous sodium methanethiolate solution (77.5ml; 23.5% w/w) is added and the mixture is stirred overnight at ambient temperature. Ethyl acetate (1 litre) is added, and the mixture is washed with aqueous potassium carbonate (2 x 500ml; 10%w/v), then with

20 aqueous sodium bisulphite (500ml; 5% w/v), dried over magnesium sulphate, filtered and concentrated under reduced pressure to give S-methyl (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate (46.3g), m.p. 169-170°C.

The present invention also includes within its scope pharmaceutical formulations which comprise an effective amount of at least one of the compounds of formula I in association with a pharmaceutically acceptable carrier or coating.

5 PHARMACOLOGICAL USES

An object of the invention is to provide a topical antiinflammatory, immunosuppressive and antiallergic steroid, or a pharmaceutical composition thereof, for the following:

topical treatment of skin conditions such as dermatitis,
10 psoriasis, sunburn, eczema, neurodermatitis and anogenital pruritis;

inhaled treatment of airways conditions such as allergy, asthma and rhinitis, chronic obstructive pulmonary disease, interstitial lung diseases and fibrosis;

15 local treatment of inflammatory bowel conditions such as ulcerative colitis and Crohn's disease; and

local treatment of conjunctiva and conjunctivitis.

The topical treatment of such conditions by steroid compounds of this invention is associated with no side-effects
20 or minimal side-effects associated with typical systemic steroid activity, such as suppression of hypothalamus-pituitary-adrenal function, mobilisation of glucose stores, collagen disorders, mineralocorticoid function, adrenal atrophy, osteoporosis and suppression of bone growth and
25 atrophy of thymic tissue.

This may be achieved by a combination of direct delivery of the steroid to the application site, and by reduced systemic activity, caused by restricted absorption or by rapid

SUBSTITUTE SHEET

in-vivo metabolism of the steroid. Thus, inactivation of the steroid can be by metabolism in the target organ or, after uptake into the general circulation, e.g. by metabolism or excretion. Such compounds are often referred to as "soft" steroids.

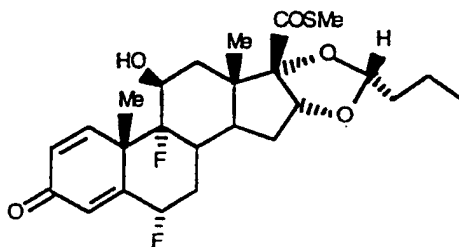
PHARMACOLOGICAL TEST SYSTEMS

Biological test results on compounds of this invention are exemplified as follows:

The following representative compound illustrates the pharmacological activity of the invention.

Compound No.: 1

Structure:



Name: S-Methyl (20R)-16 α ,17 α -butyldenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate

Specific activities for Compound No. 1:

GLUCOCORTICOID AGONIST ACTIVITY

STERIOD BINDING TO THE RAT THYMUS GLUCOCORTICOID RECEPTOR

Thymi of male adrenalectomised rats are removed and homogenised in 3-(N-morpholino)propanesulphonic acid dithiothreitol buffer, and centrifuged at 100,000g. The supernatant cytosol is used as the source of receptor.

Steroid (1-16nM in doubling dilutions) and [3 H] dexamethasone

(4nM) are equilibrated with receptor for 24 hours at 4°C. Bound [³H] dexamethasone is separated from free dexamethasone by a dextran coated charcoal technique and is quantified by liquid scintillation counting. The IC₅₀ (concentration
5 reducing [³H] dexamethasone binding by 50%) is calculated from the plot of the fraction bound against added steroid concentration.

Glucocorticoid Receptor Binding Assay, Rat Thymus activity:
IC₅₀ = 1.7nM.

10 INHIBITION OF TUMOUR NECROSIS FACTOR (TNF- α) RELEASE FROM HUMAN PERIPHERAL BLOOD MONOCYTES

Monocytes are obtained from blood samples taken from normal human donors. The leukocyte population is washed, applied to a discontinuous metrizamide gradient, and
15 fractionated by centrifugation. The monocyte-enriched interface is aspirated, the cells washed and total and differential counts performed to determine the number of monocytes. Cells are allowed to adhere to 96-well plates for 1 to 2 hours, and thereafter incubated
20 (8×10^5 monocytes/well) with the steroid for 18 hours (37°C in 5% CO₂). Cells are challenged with 10ng/ml lipopolysaccharide for 4 hours and TNF- α is assayed by use of an enzyme-linked immunosorbent assay. TNF- α quantification is performed with goat anti-human TNF- α being
25 used as the coating antibody, rabbit anti-human TNF- α as the second antibody, and goat anti-rabbit IgG horseradish peroxidase as the detection antibody. The IC₅₀ is the steroid concentration reducing TNF- α release by 50%.

SUBSTITUTE SHEET

Inhibition of TNF- α release from human peripheral blood

monocytes:

IC₅₀ = 0.16nM.

INDUCTION OF TYROSINE AMINOTRANSFERASE ACTIVITY

- 5 Rat liver H4IIE cells are cultured for 4 days until the cells are confluent. The medium is replaced by fresh medium, containing steroid under test (0-100nM) which is added to triplicate wells. After overnight incubation as above, the medium is removed and the cells are lysed and the extract
- 10 is equilibrated at 37°C with α -ketoglutarate and pyridoxal phosphate in phosphate buffer, pH 7.3, in a final volume of 1ml. Tyrosine aminotransferase activity is initiated by adding tyrosine and incubating at 37°C for 10 minutes. The reaction is stopped by adding aqueous sodium hydroxide
- 15 solution (10M). The ultra-violet absorbance of the para-hydroxybenzaldehyde is measured by a plate reader at 340nm. The maximal absorbance change achieved with the standard (dexamethasone) is used as a reference. The absorbance change for each concentration of steroid under test is
- 20 calculated as a fraction of the maximal absorption achievable and plotted against steroid concentration. The ED₅₀ is determined as the concentration causing an increase in tyrosine aminotransferase activity of 50% of the maximum achievable.
- 25 Induction of tyrosine aminotransferase activity:
IC₅₀ = 0.3nM.

ANTIINFLAMMATORY ACTIVITY IN-VIVO:

INHIBITION OF RAT LUNG OEDEMA IN-VIVO:

Test compounds are suspended in 1% carboxymethyl-cellulose/0.2% Tween 80 at double the required strength and
5 sonicated to form a suspension. This is administered
intra-tracheally (i.t.) to male rats (Sprague-Dawley strain, 6
in each group, each weighing about 350g) at 0 hours and 24
hours, with the first dose being co-administered with saline
and the second with Sephadex G200 [cross-linked dextran]
10 (10mg/ml) giving a final Sephadex concentration of 5mg/ml.
I.t. dosing is carried out under halothane anaesthesia (4% in
oxygen, at 4 litres/minute for 3 minutes). At 48 hours, the
rats are killed, final body weight is recorded, and the lungs
and thymus are removed and weighed. The doses reducing the
15 Sephadex-induced oedema and the thymus weight by 30% (ED₃₀)
are calculated. Airway selectivity is defined as the ratio
of thymus involution (ED₃₀) and inhibition of lung oedema
(ED₃₀).

Lung oedema: ED₃₀ = 0.09mg/kg

20 Thymus involution: ED₃₀ = 0.9mg/kg

Airway selectivity: 10.

INHIBITION OF MOUSE EAR OEDEMA IN-VIVO

(i) Steroids are dissolved in acetone and administered
epicutaneously to the ventral and dorsal surfaces of the right
25 ear pinna of female mice (CD1 strain, 5 in each group, each
weighing about 20g). 18 Hours later, phorbol myristate
acetate (PMA, 1.25µg/ear) in acetone is applied epicutaneously
to the right ear. The mice are killed 4 hours later, and a

5mm disc is punched out of each ear and weighed. The dose reducing PMA-induced oedema by 50% (ED₅₀) is determined from linear regression.

Inhibition of PMA-induced mouse ear oedema:

5 ED₅₀ = 0.019µg/ear.

(ii) Ovalbumin sensitised mice are challenged with antigen injected into the right ear intradermally under halothane anaesthesia (4% in oxygen, 4 litres/minute for 2 minutes) 18 hours after topical treatment with the steroids
10 [as above in (i)]. The mice are killed 1 hour later, and a 5mm disc is punched out of each ear, and weighed. The dose reducing the oedema by 50% (ED₅₀) is determined as above.

Inhibition of antigen-induced mouse ear oedema:

ED₅₀ = 0.016µg/ear.

15 In view of the results obtained when compounds of the present invention are subjected to the above tests, it can be demonstrated that valuable properties for the relief of inflammation are indicated.

In clinical practice the compounds are administered in a form suitable for the area of the body to be treated. For example, for the treatment of diseases of the respiratory system they are usually administered as aerosols or, preferably, as dry powder formulations, and for the treatment of diseases of the skin they are usually administered as creams, ointments or lotions. Such formulations are prepared by the application or adaptation of known methods such as the following.

10 PHARMACEUTICAL COMPOSITIONS

For the topical treatment of skin conditions, the steroids may be administered in a conventional pharmaceutical carrier such as creams, ointments, lotions, emulsions, solutions, foams, or the like.

15 The steroid compounds of this invention may be used for topical treatment of skin conditions, in the range of about 0.0001 to about 5%, preferably about 0.05 to about 2%, by weight of the vehicle.

For the topical treatment of allergy and asthma the
20 steroids may be administered as a dry powder, for example in a single dose inhaler or a multidose inhaler, or as a suspension or a solution in a metered dose aerosol unit or in a nebuliser, with a suitable carrier, or the like. Such devices are well known in the art and standard modes of
25 preparation may be employed or adapted.

Such formulations for inhalation typically contain from about 10 to about 4,000, preferably from about 100 to about 1,600, µg per dose.

Still further, steroids of this invention may be administered in anal or peri-anal formulations, e.g. foams, solutions or suspensions and suppositories. Such formulation techniques are well known in the art.

5 An example of a formulation as a retention enema for the treatment of ulcerative colitis (this can also be a continuous drip, i.e. a solution formulation) can be found in the specification of U S Patent 4,710,495.

10 Formulation as liposomes may be used as described in the specification of WO 92/13873.

Slow release oral formulations such as formulations for release into the intestine or colon or both, e.g. slow release tablets, may also be employed.

Such oral and anal or peri-anal formulations are
15 typically administered so as to deliver from about 0.1 to about 100, preferably from about 5 to about 50, mg per day.

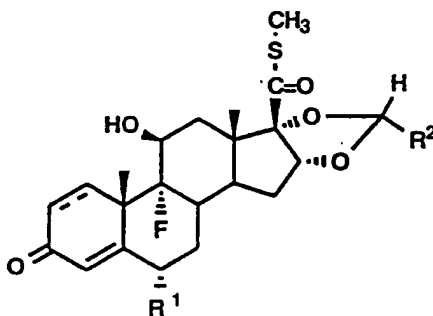
The following Composition Example illustrates pharmaceutical compositions according to the present invention.

20 COMPOSITION EXAMPLE 1

S-Methyl (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate (1.0g) (mean particle size 3.5 microns) and lactose (99g) (mean particle size 72 microns) are blended together for 30 minutes
25 in a mechanical shaker/mixer. The resulting blend is filled, to a fill weight of 25mg, into No.3 hard gelatine capsules, to give a product suitable for use, for example, with a dry powder inhaler.

CLAIMS

1. A compound of the formula:



5

where:

--- is a single or double bond;

10 R¹ is hydrogen or fluorine; and

R² is propyl or trans-prop-1-enyl and
racemic mixtures and diastereoisomers.

2. A compound according to Claim 1 where --- is a double
15 bond..

3. A compound according to Claim 1 where --- is a single
bond.

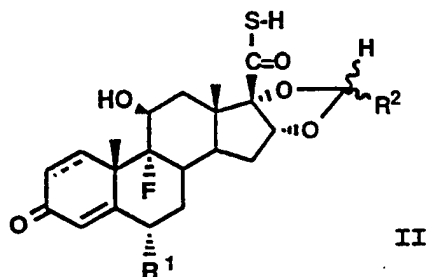
20 4. A compound according to Claim 2 where R¹ is fluoro.

5. A compound according to Claim 2 where R¹ is hydrogen.

6. A compound according to Claim 4 where R^2 is propyl.
7. A compound according to Claim 5 where R^2 is propyl.
- 5 8. A compound according to Claim 4 where R^2 is prop-1-enyl.
9. A compound according to Claim 5 where R^2 is prop-1-enyl.
- 10 10. A compound according to Claim 6 which is the (20R)-diastereoisomer.
11. A compound according to Claim 7 which is the
- 15 (20R)-diastereoisomer.
12. A compound according to Claim 8 which is the (20R)-diastereoisomer.
- 20 13. A compound according to Claim 9 which is the (20R)-diastereoisomer.
- 14 A compound according to Claim 1 which is S-methyl (20R)-16 α ,17 α -butylidenedioxy-6 α ,9 α -difluoro-11 β -hydroxy-
- 25 3-oxoandrosta-1,4-diene-17 β -carbothioate .

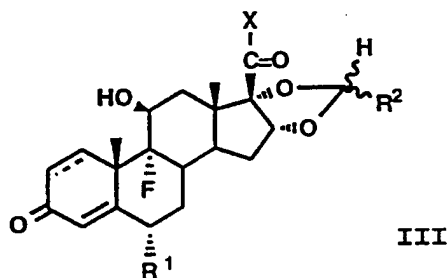
15 A compound according to Claim 1 which is S-methyl
(20R)-16 α ,17 α -[(E)-2-butenylidenedioxy]-9 α -fluoro-11 β -
hydroxy-3-oxoandrosta-1,4-diene-17 β -carbothioate.

- 5 16. A process for the preparation of a compound according to
claim 1 comprising the methylation of a compound of formula
II:



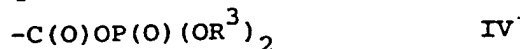
10

wherein --- , R^1 and R^2 are as defined in claim 1 or
comprising the reaction of methanethiol or an alkali metal
salt thereof with a compound of formula III:



15

wherein --- , R^1 and R^2 are as defined in claim 1 and -COX
represents a group of formula IV:



20 wherein R^3 represents an alkyl, preferably ethyl, group.

17. A process for the preparation of a compound according to claim 1 substantially as described herein.

5 18. A method for the treatment of a patient in need of an antiinflammatory, immunosuppressive or antiallergic treatment which comprises administering to said patient an effective antiinflammatory, immunosuppressive or antiallergic amount of a steroid compound of Claim 1.

10

19. A pharmaceutical composition which comprises a steroid of Claim 1 in association with a pharmaceutical carrier or coating.

15 20. A pharmaceutical composition for the treatment and/or prophylaxis of disorders associated with inflammation which comprises an antiinflammatory effective amount of a compound of Claim 1 to ameliorate said disorder.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/GB 93/02537

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C07J71/00 A61K31/56 //C07J3/00,C07J5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07J A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 004 741 (SYNTEX (U.S.A.) INC.) 17 October 1979 see the whole document ---	1,16, 18-20
A	GB,A,2 137 206 (GLAXO GROUP LIMITED (UNITED KINGDOM)) 3 October 1984 see the whole document ---	1,16, 18-20
A	THE JOURNAL OF ORGANIC CHEMISTRY vol. 51, no. 12 , 13 June 1986 , WASHINGTON, US pages 2315 - 2328 D. J. KERTESZ AND M. MARX 'Thiol esters from steroid 17-beta-carboxylic acids: carboxylate activation and internal participation by 17-alpha-acylates.' see page 2317; examples 17,18 -----	1,16,18, 20

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

8 March 1994

Date of mailing of the international search report

25.03.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Moreno, C

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB93/02537

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/GB 93/02537

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0004741	17-10-79	US-A- 4188385	12-02-80
		AT-B- 368168	27-09-82
		AU-B- 526025	16-12-82
		AU-A- 4558379	18-10-79
		CA-A- 1134345	26-10-82
		DE-A- 2912331	18-10-79
		FR-A, B 2421912	02-11-79
		GB-A, B 2018256	17-10-79
		JP-C- 1338662	29-09-86
		JP-A- 54141758	05-11-79
		JP-B- 61001038	13-01-86
		JP-C- 1374793	22-04-87
		JP-A- 60069019	19-04-85
		JP-B- 61040648	10-09-86
		SU-A- 1052161	30-10-83
		US-A- 4263289	21-04-81
GB-A-2137206	03-10-84	AT-B- 395428	28-12-92
		AT-B- 395427	28-12-92
		AT-B- 395429	28-12-92
		AU-B- 544517	06-06-85
		AU-A- 6729881	20-08-81
		BE-A- 887518	13-08-81
		CA-A- 1201114	25-02-86
		CA-C- 1205464	03-06-86
		CH-A- 644615	15-08-84
		CH-A- 651307	13-09-85
		DE-A, C 3105307	10-12-81
		DE-C- 3153379	19-11-92
		FR-A, B 2477156	04-09-81
		FR-A, B 2485542	31-12-81
		GB-A, B 2088877	16-06-82
		JP-C- 1488353	23-03-89
		JP-A- 56138200	28-10-81
		JP-B- 63037120	22-07-88
		NL-A- 8100707	16-09-81
		SE-B- 452468	30-11-87
		SE-A- 8101010	16-08-81
		US-A- 4335121	15-06-82
		US-A- 4578221	25-03-86

Information on patent family members

PCr/GB 93/02537

Form PCT/ISA/210 (patent family annex) (July 1992)